Anemia 2: Fourth year Medical Students/ 17.11.2020

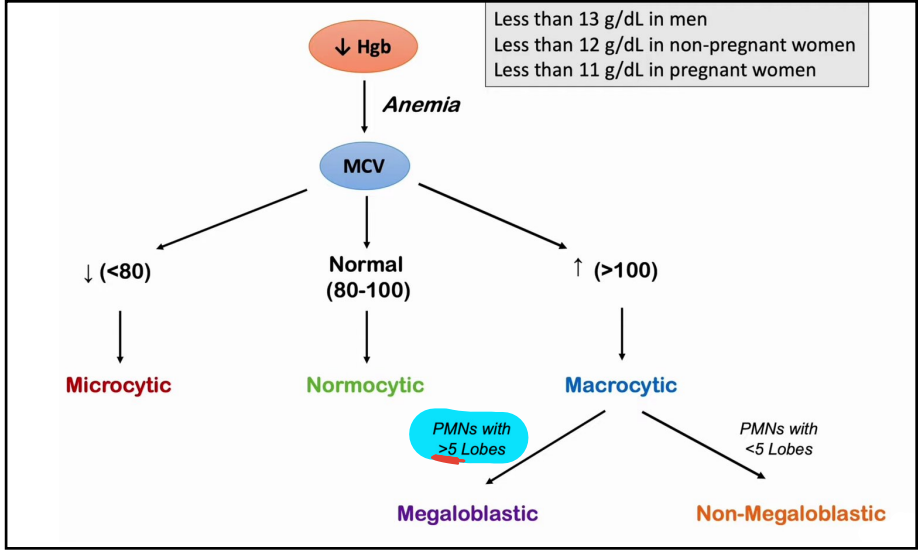
Abdallah Awidi Abbadi.MD.FRCP

Feras M Fararjeh, MD

1) Vitamin B12 deficiency
2) Folate deficiency
3) Myelodysplastic Syndrome

Modified by : بَيـان هوِيدي

*** Things to keep in mind :



✓ Anemia : Normocytic \ Microcytic \ Macrocytic

✓ Macrocytic ---> MCV is >100 FL

✓ Macrocytic Anemia can be Megaloblastic or Non-Megaloblastic

✓ The difference btw them on Blood films is having Hypersegmented neutrophils in Megaloblastic Anemia

✓ While the difference in pathophysiology is that in Megaloblastic anemia there's impairment in DNA synthesis while DNA synthesis is normal in Non-Megaloblastic anemia

✓ So Anything that causes problem in DNA synthesis leads to Macrocytic "Megaloblastic" Anemia

>>> Pathophysiology : The chromatin (nucleus) can't divide and mature while the cytoplasm matures "increases" normally > the result is large immature RBCs \ WBCs \ Megakaryocytes \ intestinal epithelium ... etc

✓ These immature blood cells will undergo earlier apoptosis > the result would be PANCYTOPENIA

>>> So Megaloblastic Anemia = Pancytopenia "not only anemia"

** This early apoptosis will cause elevated Hemolytic markers (LDH in the coming case)

✓ REMEMBER : Maturation of any cell means increase in cytoplasm volume & decrease in chromatin size

✓ As we said (chromatin isn't maturing while cytoplasm continues to mature) this is known as "nucleus cytoplasm asynchrony" and this is the Hallmark of bone marrow biopsies in Macrocytic "Megaloblastic" Anemias.

✓To sum up : Bone marrow biopsy in Megaloblastic anemia : Large immature cells , NC asynchrony , Hypercellularity (as a result of decreased mature blood cells "feedback")

✓EXTRA: Regarding the Non-Megaloblastic anemia , the pathophysiology of macrocytosis will be things other than "impaired DNA synthesis & NC asynchrony"

For example , Liver disease causes RBCs to appear large due to an increased deposition of cholesterol on the membranes of circulating RBC.

Macrocytic Megaloblastic Anemia: Causes

- The "Ds"

- **D**eficiencies
  - Folate **D**eficiency
    - Weeks to 6 Months of Storage in the liver
    - Sources: Leafy greens, fortified grains
    - Causes: 1) Reduced Dietary Intake (unlikely as daily requirement are easily met), 2) Compromised Absorption (it get absorbed in the jejunum and Ilum), 3) Impaired Metabolism, 4) Increased Utilization (= increased demand : increased production of cells as (in pregnancy , developing fetus , Hemolysis , tumors))
  - Vitamin B12 **D**eficiency
    - 3-5 Years of Storage in the liver (with 0 intake of vitamin B12 , you will need time to deplet the stores)
    - Sources: Animal products (meat , dairy products , eggs , Fortified Cereals)
    - Causes: 1) Reduced Dietary Intake (Vegans), 2) Compromised Absorption (get absorbed in the terminal Ilum)
    - ***Neurological symptoms***

- **D**NA affecting **D**rugs
  - Methotrexate
  - Azathioprine
  - Sulfa antibiotics (Septra)
  - Antivirals (Zidovudine)
  - Antiepileptics
    - Phenytoin
    - Phenobarbital
  - Metformin (due to vitamin B12 deficiency)
  - Chemotherapies
    - 5-Fluorouracil
    - Hydroxyurea
    - Cytosine
    - Cladribine
    - Capecitabine
  - Others:
    - Pyrimethamine
    - Sulfasalazine
    - triamterene
    - **Mercaptopurine**

Causes of Macrocytic Non-Megaloblastic Anemia

Endocrine
- Hypothyroidism

Genetic
- Hereditary spherocytosis
- Down's Syndrome
- Myelodysplastic Syndrome (MDS)
- Paroxysmal Nocturnal Hemoglobinuria

Reactive Causes
- Reticulocytosis

Other
- Pregnancy
- Liver Disease
- Alcoholism (even without liver disease)
- **Smoking**

Case

65 yr old male had gradual onset of "odd" behavior with psychotic symptoms, irritability and parasthesia in hands and feet

He was noticed to have imbalanced gait.

Examination showed loss of vibration and proprioception in lower limbs

All of these are neurological finding indicating vit.B12 defeciency as a cause of the anemia

cobalamin = vit.B12

Laboratory tests

He's severely anemic — Macrocytosis

Hb **5** g/dl, MCV **112**,

Anemia of low production

Retics (corrected)0.**009** >> very very low

Low (AKA : leukopenia) — Thrombocytopenia | Low

Normal : 4.5 to11.0 × 10⁹ / L — WBC 3.3k, Platelets 112k — Normal Platelets count : (150K - 450K)/ microliter

high (remember it's a hemolytic marker)

Normal serum B12 : more than 200 picograms

Normal : up to 450 — LDH 1900. Serum B12: 30 pg/ml.

till here , it's Macrocytic Anemia caused by vit.B12 def which is Also the cause of the neuropsychiatric manifestations mentioned in the previous slide

But what's the cause of this Vit.B12 def ?

Intrinsic factor Autoantibodies +ve — IF Ab +PCA+ — parietal cells Autoantibodies +ve

Low stomach acidity caused by the parietal cells damage — **Achlorhydria+**

These are indicating the cause which is pernicious anemia

When doing upper endoscopy , the biopsy showed Atrophic gastritis — Gastric Bx atrophic gastritis.

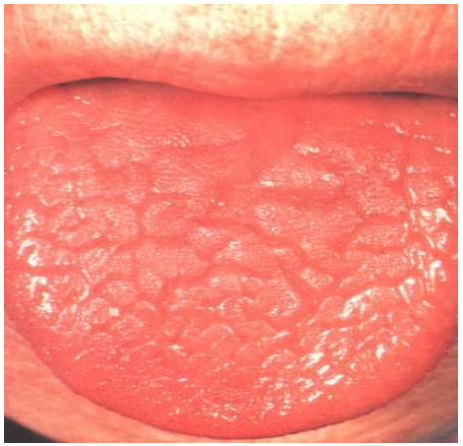
** Pernicious Anemia is Autoimmune disease where u have Autoantibodies against The intrinsic factor or the parietal cells (responsible for IF synthesis) leading to decrease in vitamin B12 absorption , it's the most common cause of vitB12 def

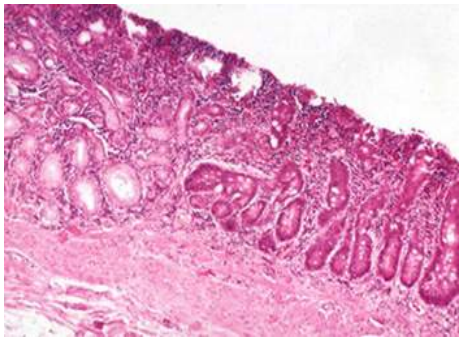
Remember : parietal cells (AKA oxyntic cells) produce the gastric acid and the intrinsic factor

1-4 >> found in any vit.B12 def of any cause Not specific for pernicious anemia

Physical And Lab

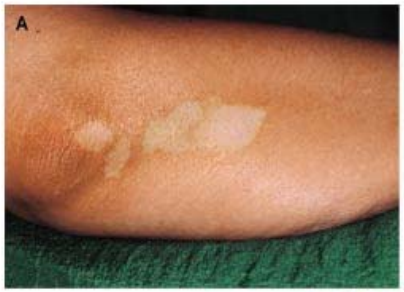
Red Beefy Tongue

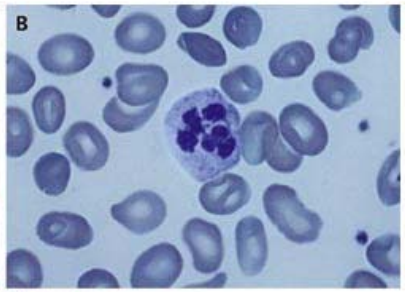


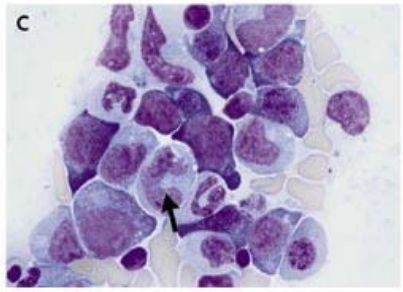


Autoimmune association (maybe also with Hashimoto's or DMT1)

Vitiligo







Due to inflammation

Oxyntic G. mucosa atrophy

Blood film :

Macro-ovalocytes.

Hypersegmented neutrophils

Hypersegmented neutrophils >> more than 5 segments

BM biopsy :

Megaloblasts

= Large immature cells with NC asynchrony

Pathogensis of Pernicious Anemia (PA)

1-PA is the end-stage of Atrophic Body Gastritis (ABG) causing oxyntic gastric mucosa damage: achlorhydria.

2-It is considered an autoimmune disease (AID).

3-AID theory is based on the presence of parietal cell and/or intrinsic factor autoantibodies

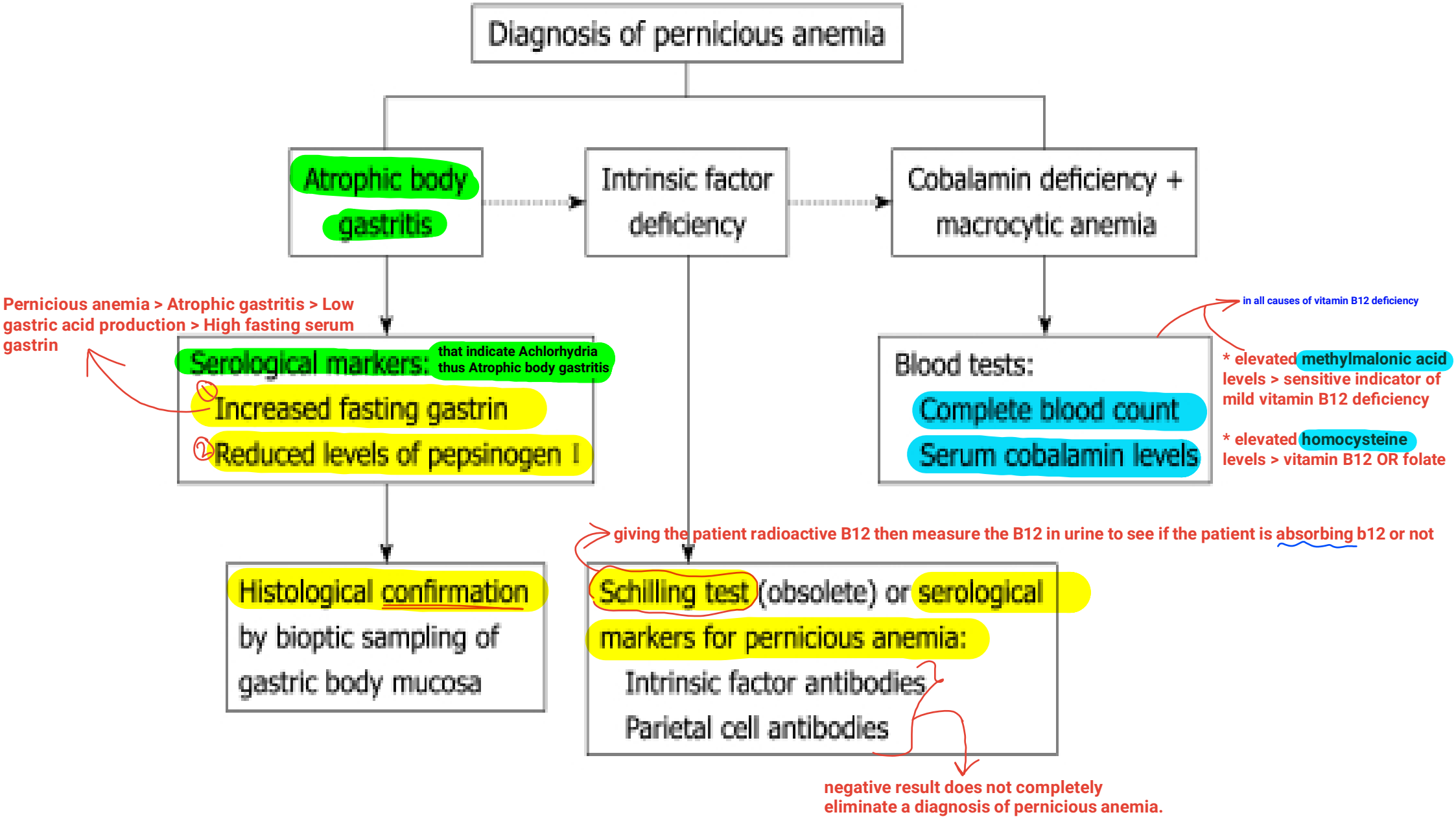
- ✓ Parietal cells Antibodies can be present in normal subjects
- ✓ what is more imp for the pathogenesis is the IF-AB

Frequent association with other autoimmune disorders: autoimmune thyroid disease (ATD), type 1 diabetes, and vitiligo

Remember :

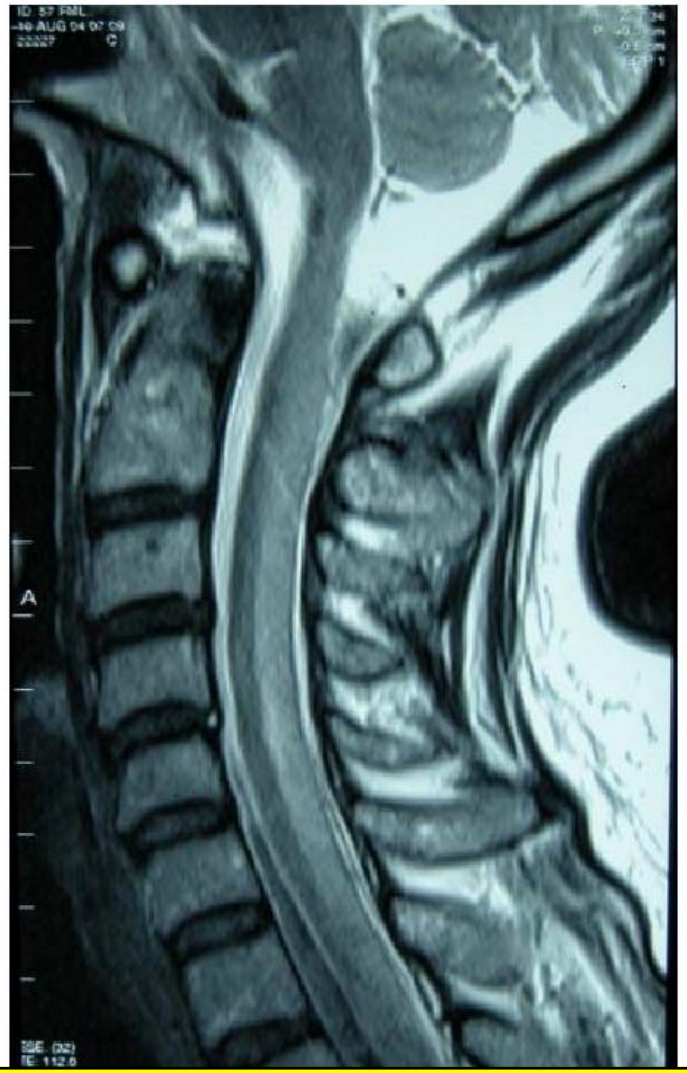
✓ **Gastrin is secreted by G-cells to increase gastric acid production by the parietal cells**

✓ **pepsinogen is a digestive enzyme secreted by the chief cells as a proenzyme and then converted by gastric acid in the gastric lumen to the active enzyme pepsin**

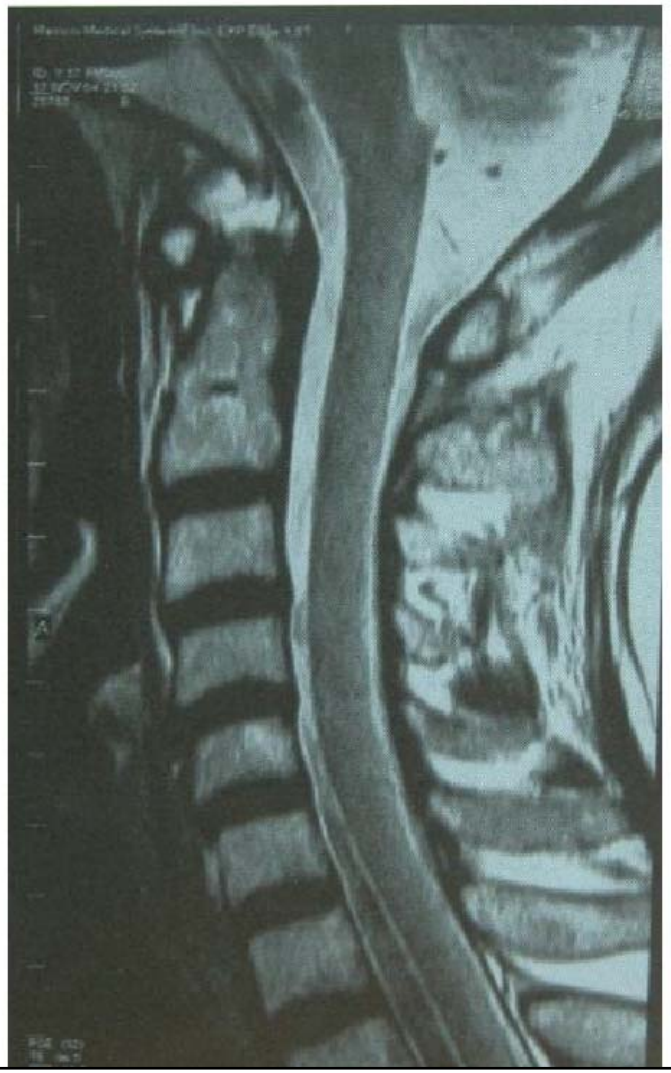


***** vit.B12 is important for myelination**

A-Before therapy



B-Post-therapy



A- Hyperintense in cervical region

B- corrected

Vit.B12 def effect on nervous system :

1) peripheral neuritis (most common)

2) Subacute Combined Degeneration of the Spinal Cord (most serious) affecting the posterior and both lateral columns of the SC

3) Dementia

loss of proprioception , vibration sensation & 2 points discrimination

muscle weakness , spasticity , ataxia (cerebellar tract)

Subacute Combined Degeneration of Spinal Cord

once you suspect that the patient has vitamin B12 deficiency you have to start treatment with IM injections ASAP without waiting for the results in order to reverse the damage that occured and prevent further damage

Case 2 : Treatment & Monitoring

No Blood Transfusion

you can't give it orally in patients with pernicious anemia or other causes of malabsorption for vitB12

Vit B12 IM injections daily 7-10 days. Then monthly lifelong.

Careful monitoring of response

Careful monitoring for thyroid function & DM

Vitamin B12 and Hemolytic markers

✓ NOTE : High indirect Bilirubin , high LDH , high haptoglobin and high reticulocytic count >> are signs of Hemolysis (AKA Hemolytic markers)
✓ These markers are elevated in Megaloblastic anemia due to earlier apoptosis of the cells
✓ In VERY sever vit.B12 def apoptosis and Hemolysis will occur to the cells in the BM before getting out to the peripheral blood, and this is known as (Medullary Hemolysis) and it's a sign of sever vitamin B12 deficiency.

Response to Treatment

Reticulocytosis in 3-4days, peak 5-10 days

Rise in Hgb concentration within 10 days and normalization in 8-10 weeks as well as correction of MCV.

Fall of serum LDH levels within 2 days

Hypersegmented PMN disappear in 10-14 days

✳Watch closely for severe hypokalemia during early response.

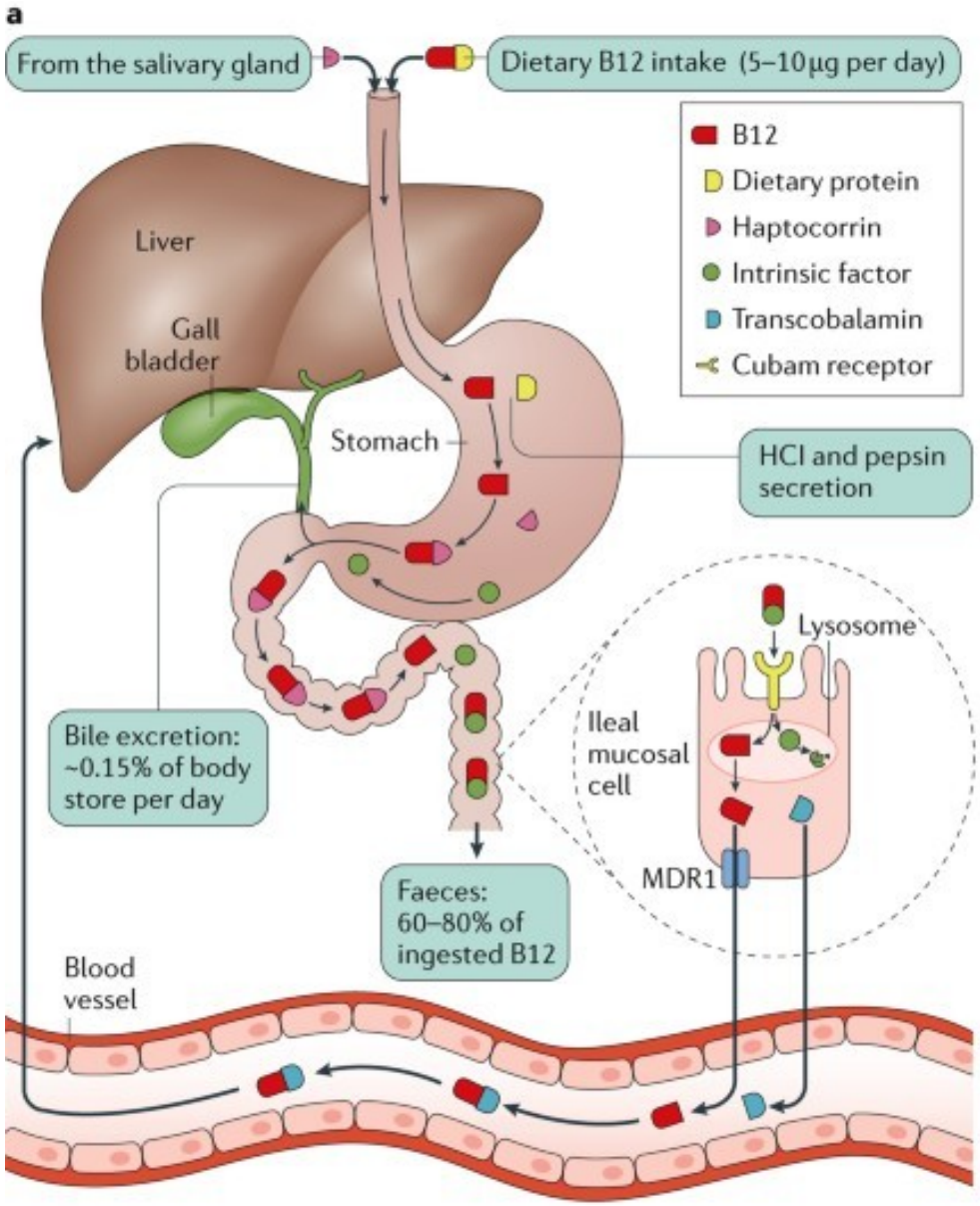
WHY ??
>> When giving cyanocobalamin (vit.B12) as a treatment, there is an increase in erythrocyte metabolism, which leads to hypokalemia. Therefore as the anemia is corrected , serum potassium should be monitored carefully during therapy.

Megaloblastic changes disappear within 2 days

↳ will start to change within hours

To understand other causes of vitamin B12 deficiency you have to understand it's absorption :

✓ We need pepsin to cleave vitamin B12 from the dietry protein
✓ We need high acidity to convert pepsinogen to pepsin
✓ We need IF produced by the parietal cells
✓ We need normal Ilum (Terminal Ilum is the site of vitamin B12 absorption)



Other causes of cobalamin deficiency

Gastrectomy/ gastric sleeve operations

Corpus-predominant *H pylori* gastritis — low acidity

Long-term proton pump inhibitor therapy

Ileal disease or resection

- **Crohn's disease**

Blind loop syndrome

- **medications such as metformin**

Fish tapeworm

problem in absorption site

- **pernicious anemia** — No IF

Severe pancreatic insufficiency — low pepsinogen

Decreased intake due to vegetarianism

EXTRA : Metformin
More recent theories indicate that absorption of the B12 intrinsic factor complex uptake by cell membrane receptors in the ileum is known to be " calcium-dependent" and metformin interferes with the calcium-dependent membrane action. This interference therefore affects B12 absorption and reduces B12 levels.

Other causes of macrocytic anemia

Folate deficiency

Drugs (e.g. metformin, methotrexate, azathioprine, 6-mercaptopurine)

erythropoiesis: hemolysis, response to hemorrhage)

Liver disease (alcoholic, cirrhosis, poor dietary intake)

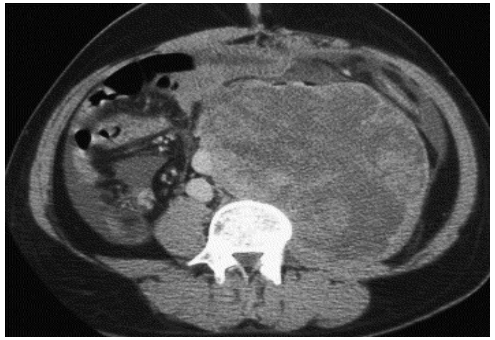
Hypoplastic anemia, myelodysplastic syndrome

Case

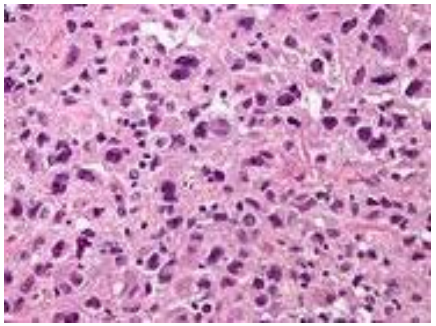
65 yr old male had "anemia syndrome" over the last 6 weeks. He noticed abdominal swelling and weight loss. He had mild fever and night sweats for 2 weeks. No neurological symptoms or signs.

Anemic Macrocytosis Normal Slightly low
Hb 9, MCV 106, WBC 5.3, Plt 142, Retics
anemia of reduced production Elevated
(corrected)0.1%. Serum B12 normal. LDH 1100. serum
Low Normal : up to 450
folate was 0.2 Normal : 3-4

Abdominal Ct
Showing retroperitoneal mass



Biopsy (undif. sarcoma)



** This is a case of Macrocytic Megaloblastic Anemia caused by folate deficiency
** The cause of folate deficiency is increased demand caused by malignancy

Causes of Folic acid deficiency

1. Inadequate intake → Hard as daily requirement are easily met

- diet lacking fresh, uncook food; chronic alcoholism, total parenteral nutrition,

→ As in ICU patients

2. Malabsorption

- small bowel disease (sprue, celiac disease,)
- alcoholism

3. Increased requirements:

- pregnancy and lactation
- infancy
- chronic hemolysis
- **malignancy**
- hemodialysis

4. Defective utilisation

Drugs:folate antagonists(methotrexate, trimethoprim, triamteren), purine analogs (azathioprine), primidine analogs (zidovudine), RNA reductase inhibitor (hydroxyurea), miscellaneous (phenytoin, N_2)

Case 2 B:
Treatment and follow-up

Treat the original Cause

Folic acid is the synthetic form of folate

Can also be administered parentally (IV) in sever deficiency causing pancytopenia

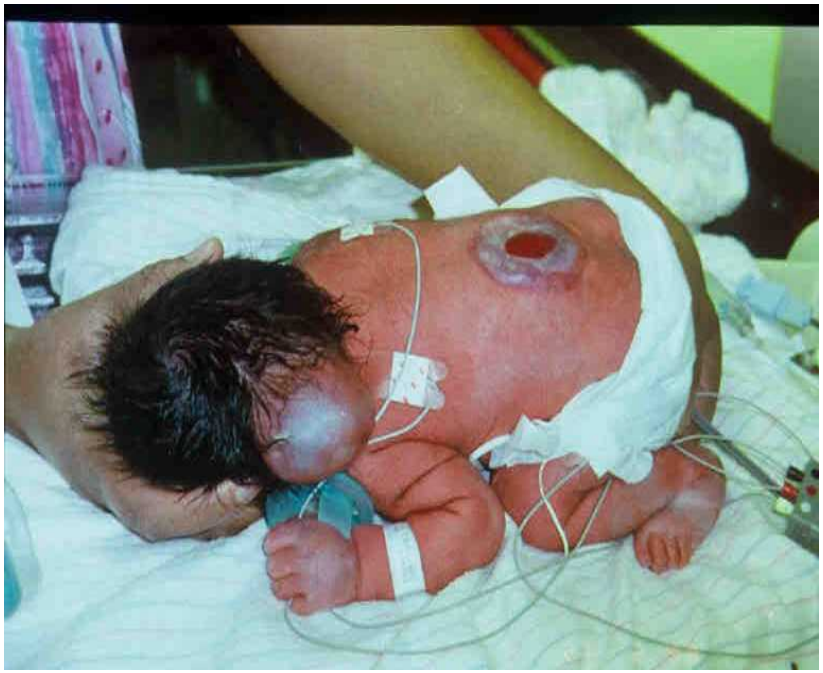
Oral administration of folic 5 mg x2daily, for 3 months, and maintenance therapy if it is necessary.

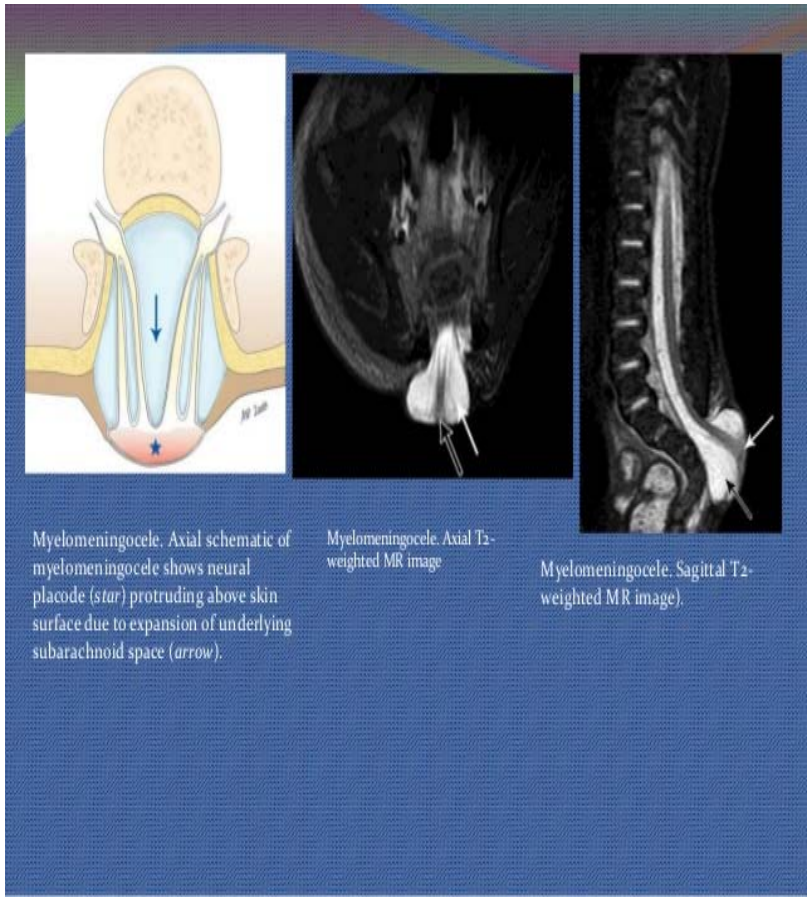
Retics after 5-7 days.

Correction of anaemia after 2 months therapy.

✓✓ When u start treating folate def with folic acid , check vitamin B12 levels first in order not to create vit.B12 def by the treatment (as erythropoiesis will increase when giving folic acid)

Folic acid has role in neural tube closure in foetus, a pregnant woman should have enough folate to protect her foetus from having neural tube defects





Myelomeningocele. Axial schematic of myelomeningocele shows neural placode (*star*) protruding above skin surface due to expansion of underlying subarachnoid space (*arrow*).

Myelomeningocele. Axial T2-weighted MR image

Myelomeningocele. Sagittal T2-weighted MR image).

* MDS : Pancytopenia + hypercellular BM (full with dysplastic cells)
*Aplastic Anemia : Pancytopenia + Hypocellular BM

Case

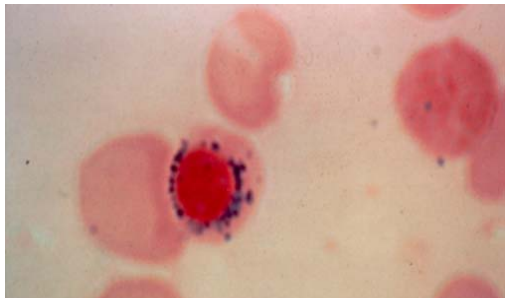
48 yr old lady presented with "anemia syndrome" for 3 months. She was found to have splenomegaly. Hb 8g, MCV 107fl, WBC 3.6, plt 95k, retics 0.6%.LDH350

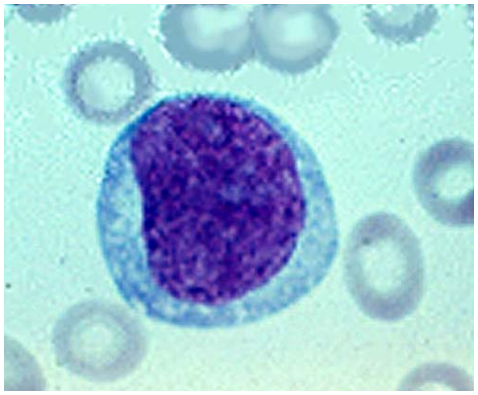
Anemic
Macrocytosis
Low
Low
Anemia of low production
Normal
Pancytopenia

BM: ringed sideroblasts, blasts 8%. Cytogenetics by FISH 11 q del.

deletion in the long arm of chr.11

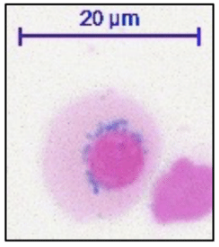
Normally Blasts Not more 5% in the bone marrow
if more but less than 20% >> MDS
if more than 20% >> leukemia





Pancytopenia + cytogenetic aberration +20> blasts >5

Diagnosis: MDS:
RARS/RAEB type I with ring sideroblasts



Sideroblasts : nucleated erythroblasts with granules of iron accumulated in the mitochondria surrounding the nucleus.

What are MDS?

it's Mainly a disease of elderly age group

- MDS: a spectrum of heterogeneous malignant hematopoietic stem cell disorders characterized by ineffective and dysplastic changes in BM with
  - ineffective haemopoiesis- dysmorphic cells in blood
  - Variable cytopenia- frequent possible progression to aml
- MDS may occur

  a-de novo: primary MDS b-as a result of haemopoietic stem cell injury: secondary or treatment-related MDS
- MDS is associated with significant morbidity and mortality due to
  - cytopenias
  - impaired quality of life
  - risk of transformation to AML

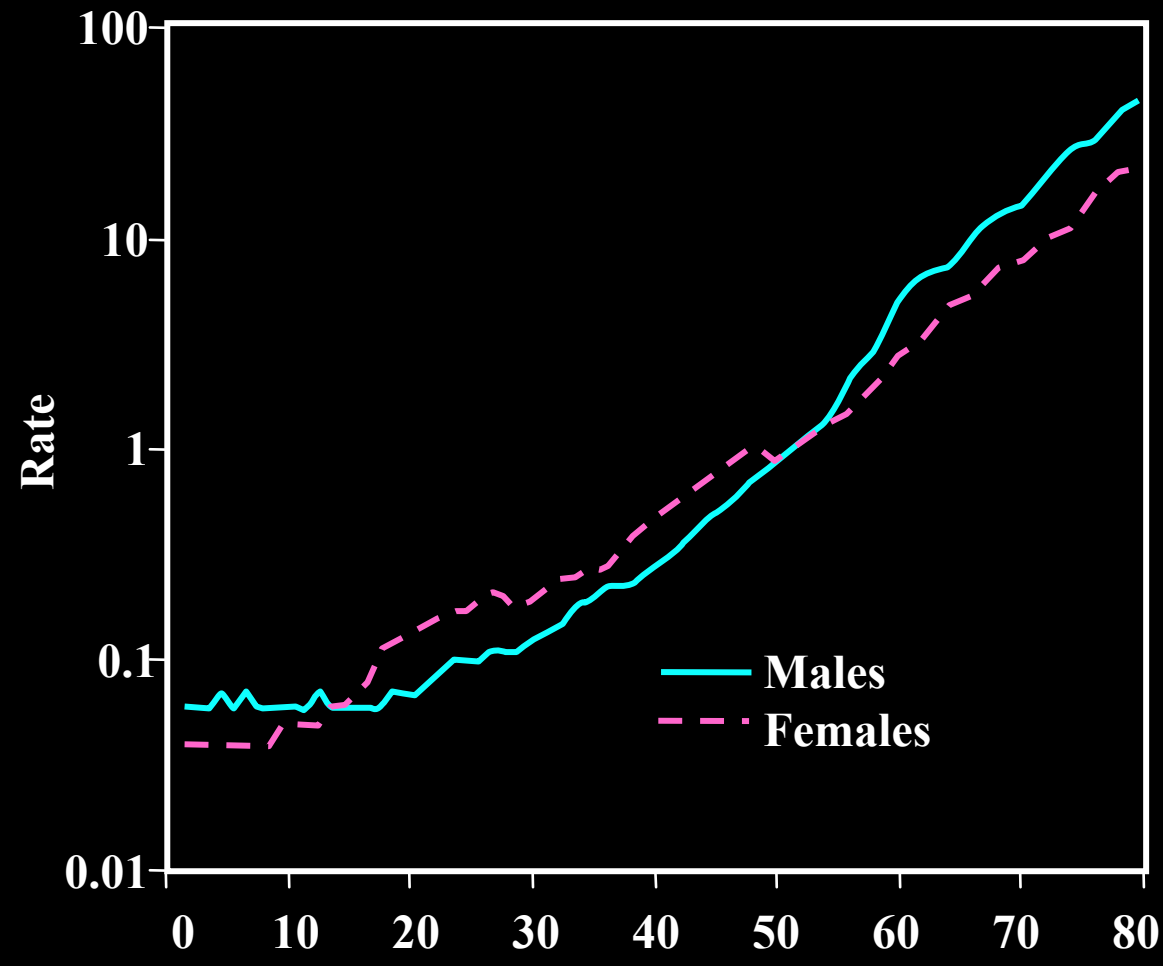
Epidemiology of MDS

- Epidemiology of MDS
  - common bone marrow disorder
  - the overall incidence is approximately 5 per 100,000 in the general population
  - peak incidence occurs at 60–90 years of age

    > 20 per 100,000 at 70 years of age
- Typical MDS patient
  - elderly
  - slight male preponderance
  - approximately 50% have a cytogenetic abnormality

Age-related Incidence of MDS

Leukaemia Research Fund [1984-1993]



McNally RJQ et al. *Hematological Oncology* 1997. 15:173-189,
Cartwright RA,et al. Leukaemia Research Fund, 1997. http://www.lrf.org.uk
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Pathogenesis

Poorly understood
Clonal process, thought to arise from single hematopoietic progenitor cell that acquired multiple mutations
Global hypomethylation with concomitant hypermethylation of gene-promoter regions.
Mutation in genes that encode enzymes, such as TET2, IDH1, IDH2
As role for immunosuppressive agents, suggest immune system implicated in myelosuppression and/or marrow hypocellularity

Clinical features in MDS

- Anaemia
  - > 80% of patients with MDS are anaemic at diagnosis
  - Granulocytopenia
  - 50–70% of patients
  - predisposition for infections
- Thrombocytopenia in 30% of patients
- In MDS
  - chronically low Hb levels associated with cardiac remodelling and increased incidence of heart failure

Diagnosing MDS

Cytopenia(s) → suspect MDS

Recommended evaluations

- History and physical examination
- Complete blood, platelets, differential, and reticulocyte count
- Examination of peripheral smear
- Bone marrow aspiration with iron stain + biopsy + cytogenetics

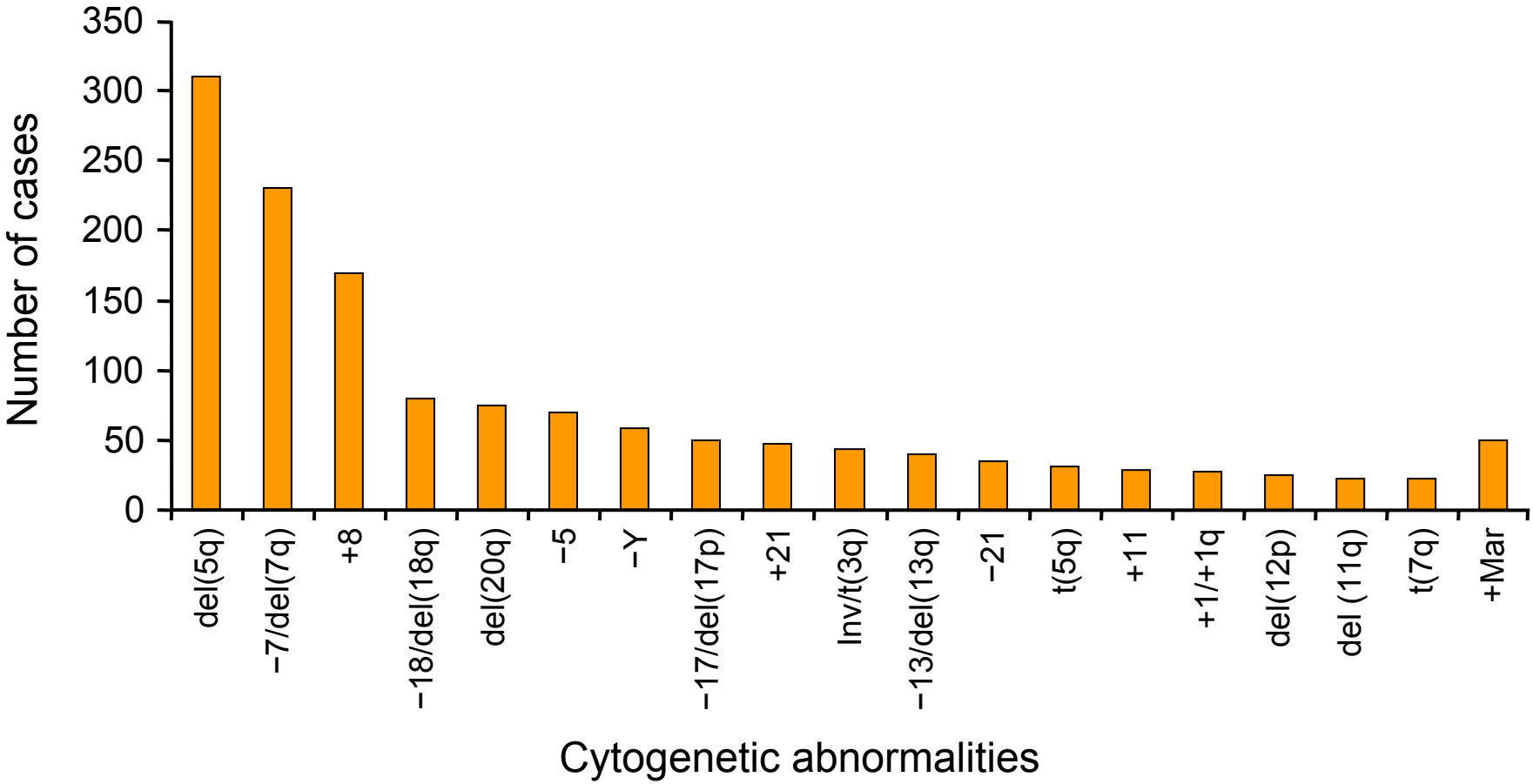
- Serum erythropoietin (prior to RBC transfusion)
- RBC folate and serum vitamin B_{12}
- Serum ferritin
- Documentation of transfusion history

Diagnosis of MDS based on morphologic and clinical criteria

Subtypes of MDS: WHO classification

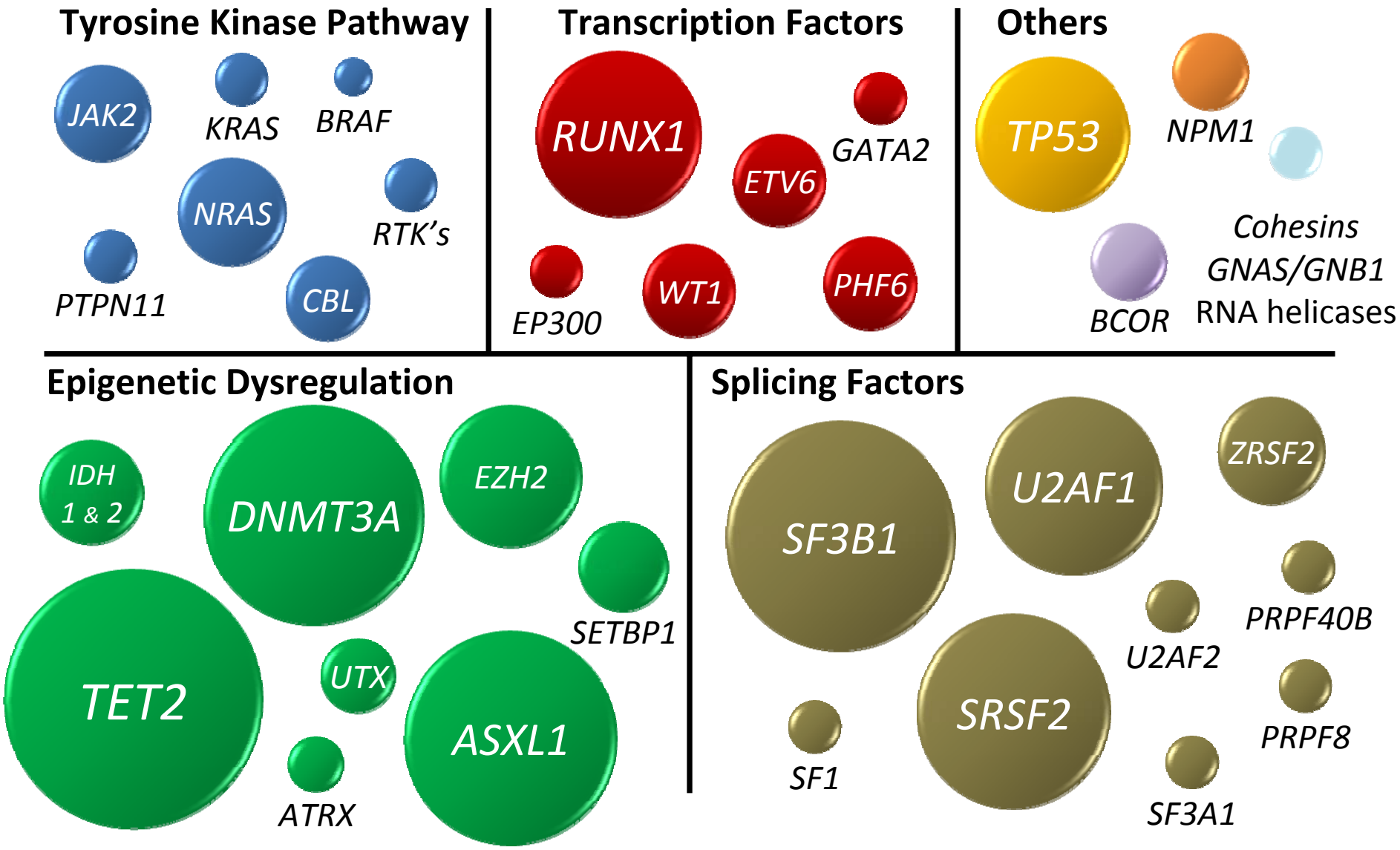
Disease	Blood findings	Bone marrow findings
Refractory anaemia (RA)	Anaemia No or rare blasts < 1 × 10^9/L monocytes	Erythroid dysplasia only < 10% grans or megas dysplastic < 5% blasts, < 15% ringed sideroblasts
Refractory anaemia with ringed sideroblasts (RARS)	Anaemia No blasts	Erythroid dysplasia only < 10% grans or megas dysplastic ≥ 15% ringed sideroblasts, < 5% blasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenias (bicytopenia or pancytopenia) No or rare blasts No Auer rods, < 1 × 10^9/L monocytes	Dysplasia in ≥ 10% of cells in two or more myeloid cell lines < 5% blasts in marrow, no Auer rods, < 15% ringed sideroblasts
Refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS)	Cytopenias (bicytopenia or pancytopenia) No or rare blasts No Auer rods, < 1 × 10^9/L monocytes	Dysplasia in ≥ 10% of cells in two or more myeloid cell lines ≥ 15% ringed sideroblasts, < 5% blasts, no Auer rods
Refractory anaemia with excess blasts-1 (RAEB-1)	Cytopenias < 5% blasts No Auer rods, < 1 × 10^9/L monocytes	Unilineage or multilineage dysplasia 5–9% blasts, no Auer rods
Refractory anaemia with excess blasts-2 (RAEB-2)	Cytopenias 5–19% blasts Auer rods ±, < 1 × 10^9/L monocytes	Unilineage or multilineage dysplasia 10–19% blasts, Auer rods ±
Myelodysplastic syndrome, unclassified (MDS-U)	Cytopenias No or rare blasts, no Auer rods	Unilineage gran or mega dysplasia < 5% blasts, no Auer rods
MDS associated with isolated del(5q)	Anaemia < 5% blasts Platelets normal or increased	Normal to increased megakaryocytes with hypolobulated nuclei < 5% blasts, no Auer rods, isolated del(5q)

Frequencies of the most common cytogenetic anomalies in patients with MDS

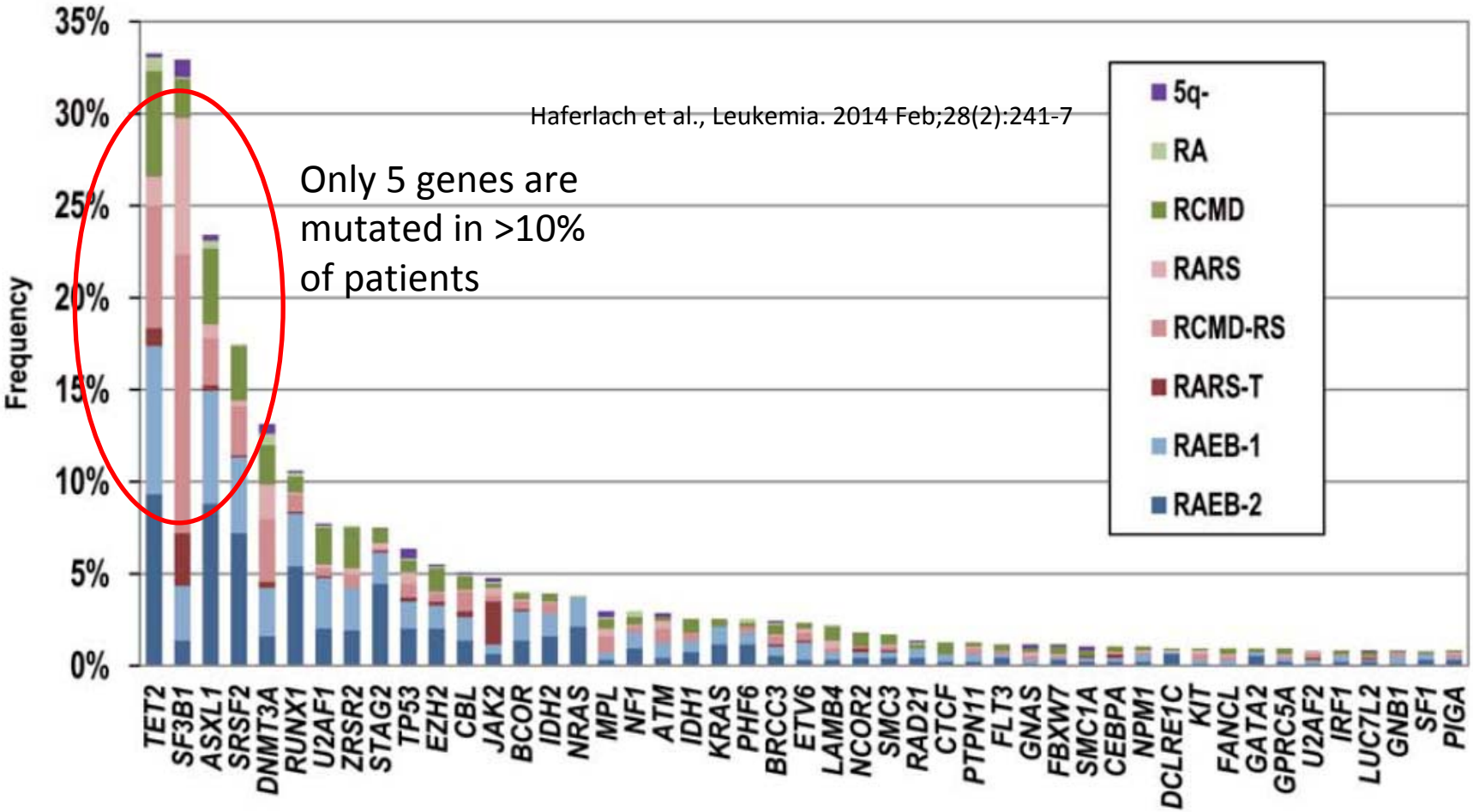


Haase D, et al. Blood. 2007;110:4385-95.

Point Mutations in MDS



Many mutations are very rare



WHO classification-based Prognostic Scoring System (WPSS)

Variable	0	1	2	3
WHO category	RA, RARS, isolation 5q−	RCMD, RCMD-RS	RAEB-1	RAEB-2
Karyotype*	Good	Intermediate	Poor	–
Transfusion requirement	No	Regular	–	–

*Karyotype: **good**: normal, -Y, del(5q), del(20q); **poor**: complex (≥ 3 abnormalities), chr 7 anomalies; and intermediate: other abnormalities.

Score	WPSS subgroup	Median survival (months) Italian cohort	Median survival (months) German cohort
0	Very low	103	141
1	Low	72	66
2	Intermediate	40	48
3–4	High	21	26
5–6	Very high	12	9

Malcovati L, et al. J Clin Oncol. 2007;25:3503-10.

Case 2 C

WPSS

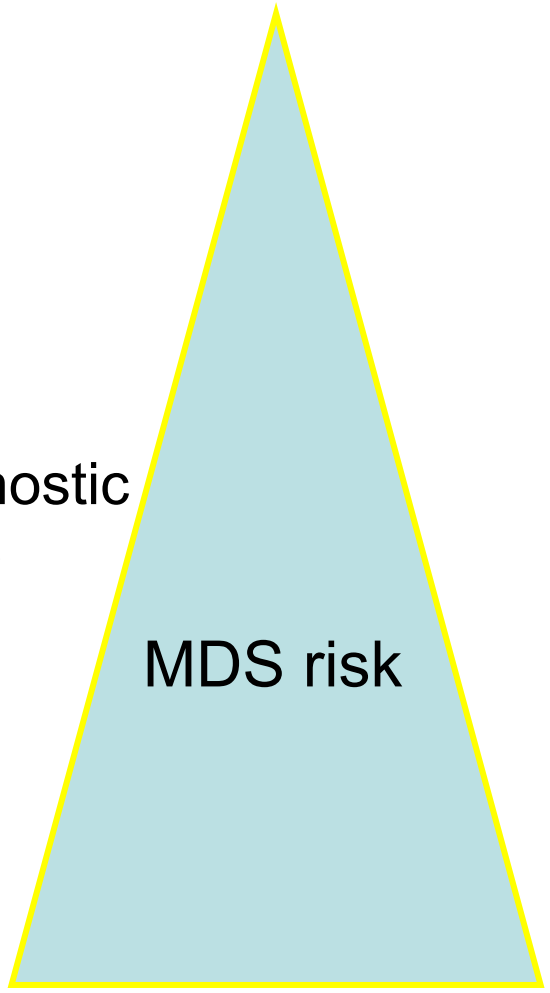
WHO category =2

Cytogenetics intermed. = 1

Bld Trx = 0

Total score 3. ms 21-26 months

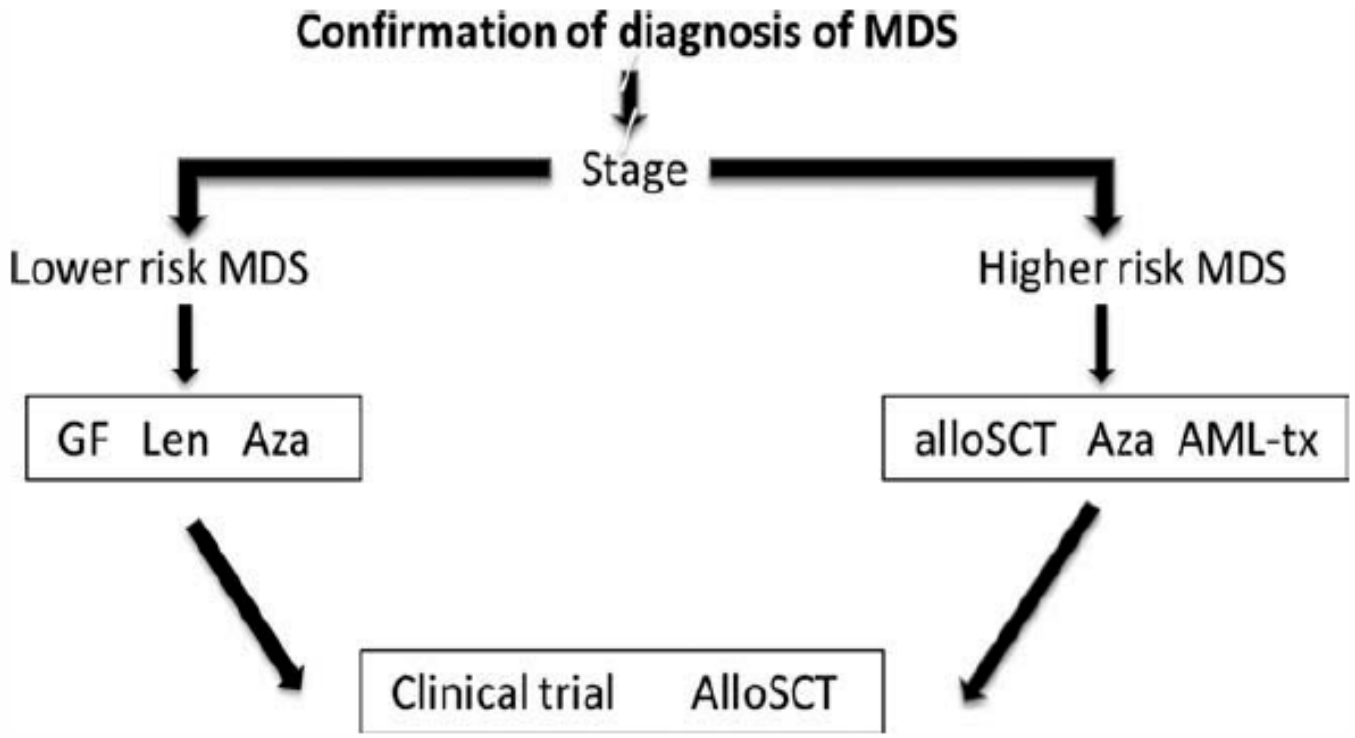
MDS: therapeutic options



- “Best supportive care”, including iron chelation
- Haemopoietic growth factors
- Immunosuppressive treatment
- Differentation induction
- Immunomodulatory drugs
- Arsenic trioxide
- Low-dose chemotherapy
- Epigenetic treatment
- Intensive chemotherapy
- Allogeneic SCT

SCT = stem cell transplantation.

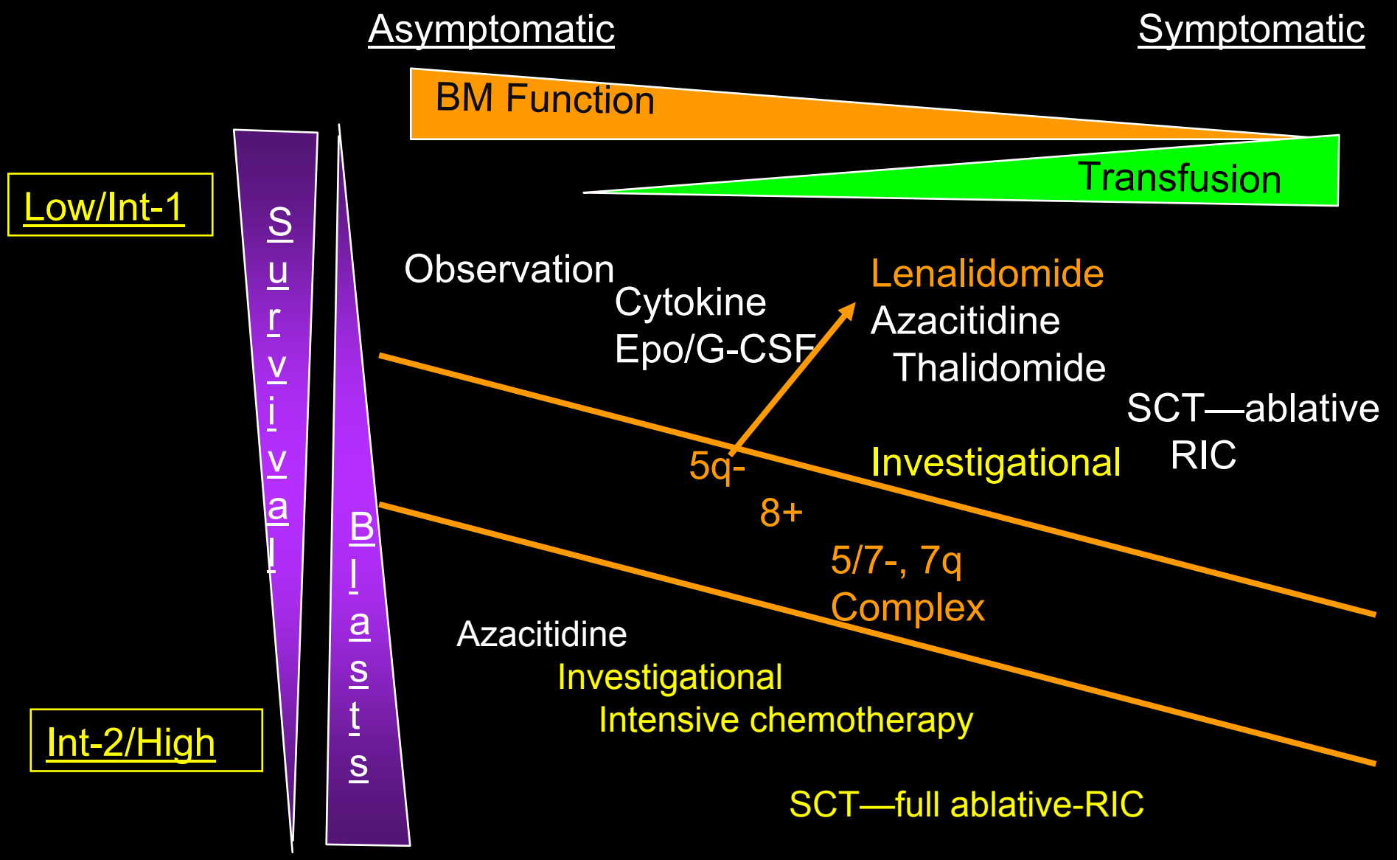
proposed general treatment algorithm



Treatments can be complicated by advanced age, comorbidities, chronicity of the disease.

Garcia-Manero, G. Am J Hematol. 2012.

Treatment Algorithm for Patients With MDS



RIC = reduced intensity conditioning.
From Silverman. In: Holland et al, eds. *Cancer Medicine*. 7th ed. BC Decker; 2006, .